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Degradation of the chemotherapy drug 5-Fluorouracil on medical silver surfaces

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The catalytic effect of a commercial grade silver surface on the degradation of the chemotherapy drug 5-Fluorouracil was studied using X-ray Photoelectron Spectroscopy and X-ray Absorption Spectroscopy. Quantitative analysis of the XPS data show that 100 % of the drug has degraded at body temperature, and that the fluorine atom has left the molecule, presumably leading to the formation of hydrofluoric acid. Remarkably, these results are even more severe than that reported by Mazzola *et al.* [2D Materials **2**, 025004 (2015)] for 5-Fluorouracil on a monocrystalline silver surface, in which case 80% of the drug reacted at body temperature. We speculate that the increased reactivity observed, may be due to a competing reaction pathway which results in the formation of HF. Our measurements indicate that polycrystalline silver alloys, which are typically used in medical equipment, can result in severe complications for the patients receiving the treatment of cancer. In this respect, high quality graphene, proposed by Mazzola *et al.* [2D Materials **2**, 025004 (2015)], is still the best alternative among possible coating materials for the catheters used in delivering 5-Fluorouracil.

The number of new cancer cases reported each year is increasing. In 2012, 14.1 million new cases were reported worldwide, and the *World Cancer Report 2014* predicts that the number will be close to 25 million a year in 20 years time [1]. Chemotherapy drugs are commonly used in cancer treatment, and in many cases such drugs are delivered intravenously by the use of a central venous catheter and metal connections. The catheters used for this purpose are tubes usually made from plastic, which may be coated with argentic alloys to prevent them from a possible deterioration due to interaction with the drug and with bodily fluids [2, 3]. 5-Fluorouracil (5-Fu) is one of the most used chemotherapy drugs in the treatment of cancer, the most used for treating colorectal and breast cancer [4]. The 5-Fu molecule is almost identical to the nucleobase uracil, except that a hydrogen atom is replaced by a fluorine atom. By inhibiting the process of DNA replication, the 5-Fu affects the reproduction of cancer cells, eventually leading to their death.

The interaction between the cancer drug and the catheter may play a central role in the reported incidences of catheter complications, including ruptures [5, 6]. Mazzola *et al.* [7] have studied the chemical changes in 5-Fu when in contact with high quality Ag single crystal. The results indicate that the drug degrades, leading to the formation of hydrofluoric acid. Although monocrystalline silver provides useful indications of what happens inside a catheter, the pristine surface quality is far from what can be expected from real medical equipment. The quality, flatness and the different grade of the material used are all relevant to characterize a reaction as these factors play a significant role for the catalytic activity of a certain system. In particular, in this work we show that the efficiency of the molecule deterioration is higher for lower quality of the sample surface. The silver surface used in this experiment is close to hospital grade: the sample is a polycrystalline alloy containing 95 % silver.

Surface contamination can certainly contribute to affect the bonding between the 5-Fu molecule and the silver surface, thus two different levels of cleaning were examined. The main objective is to compare how the 5-Fu molecule behaves on the model monocrystalline silver surface —using data provided by Mazzola *et al.* [7]— to how it behaves on a more realistic polycrystalline Ag surface. The latter is similar to the silver-based materials which are generally used in hospitals and patient environments, providing a relevant study for current medical situations which involve the use of Ag-based materials.

Two silver samples were cut from a sheet of polycrystalline silver (95 % of Ag). One surface was cleaned by rinsing it with ethanol (as would be done in a hospital environment) and the other was cleaned using oxygen plasma etching, which is expected to remove a larger amount of contamination. The samples were mounted on the same sample holder, then heated up to 500 °C in ultra high vacuum to further remove bulk contamination. After this cleaning procedure, the 5-Fu was thermally evaporated onto the samples

whose temperature was stabilized at −170 °C, such to lower the thermal energy available for a reaction, ensuring that the molecules stick to the surface. The samples were then measured cold, and whilst being warmed to body temperature.

X-ray Photoelectron Spectroscopy (XPS) and X-ray Absorption Spectroscopy (XAS) measurements were performed using synchrotron radiation at beamline D1011 at the MAX IV laboratory (Lund, Sweden). The powerful combination of these techniques allows to uncover the elemental composition of the samples, their atomic configuration and ultimately the sign of possible reactions between the argentic buffer layers and 5-Fu. It is well known that the sampling depth in and XPS measurement is dependent on the kinetic energy of the emitted electrons [8]. In our data acquisitions, the photon energy was chosen differently for each core level, such that the photoemitted electrons would have a comparable kinetic energy (approximately 160 eV), thus the same escape depth (corresponding to a mean free path of approximately 0.5 nm). As shown schematically in Fig. 2 (a), the depth sensitivity depends on the energy of the photons used.

The XPS measurements presented in this work have undergone a Shirley-plus-linear background subtraction and each component of the core level peaks has been fitted by a Voigt function. The number of components and free parameters were chosen based on the stoichiometry of the 5-Fu molecule (F : N : C : O = 1 : 2 : 4 : 2), on the basis of reducing χ^2 [9, 10], i.e. maximizing the likelihood function, and on physical interpretations. All Voigt-shaped components in the models have the same full-width-at-half-maximum (FWHM), set equal to the smallest recorded FWHM for the samples (1.57 eV, based on the N1s peak at 38 °C, cf. Fig. 3b). The height and area of the components used to fit a single photoelectron peak are fixed to be the same (e.g. Fig. 2). This gives the best agreement between data and model. All data presented in this work have undergone an intensity renormalization accounting for the beam current and the cross sections of the elements investigated [11, 12].

To monitor the degree of surface contamination, reference spectra were acquired from the nominally clean samples before depositing the 5-Fu. Such spectra are shown in Fig. 1 (a), with their quantitative analysis in Fig. 1 (b) showing that the main sample contamination can be attributed to carbon. Assuming that the contaminating species are homogeneously distributed on the surface the C amount can be considered to form a ≈ 2 nm thick film onto the top of both the differently cleaned Ag samples.

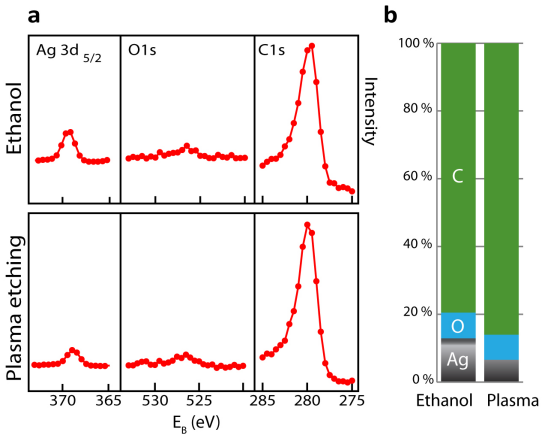

Figure 1. (a) XPS measurements of the Ag $3d_{5/2}$, O1s and C1s core levels of polycrystalline Ag samples cleaned using ethanol and plasma etching, respectively. Here, the kinetic energies of the electrons are in the range 475 eV - 720 eV, corresponding to a mean free path of approximately 0.8 nm. (b) Contamination levels relative to total signal from the three peaks. We estimate the contamination thickness to be ≈ 2 nm for both samples.

The deposition of 5-Fu was carried out by thermally evaporating a ≈ 4 nm thick film (see Fig. 2 (b) onto the Ag samples. Such a film thickness is enough to be considered bulk-like within the probing depth of our measurements, hence the XPS signal originates far from the Ag interface. This provides reference spectra for the unreacted 5-Fu. The data acquisitions have been fitted with multiple components, probably arising from inhomogeneity in the sample (for example, unequal doping). However, this does not affect the quantitative analysis.

From the fit results we find the stoichiometry of the material to be F : N : C : O = 0.9 : 2 : 4.1 : 1.7 (normalized to N ≡ 2), in agreement with the expected stoichiometry of an intact molecule. Equally important, it is comparable to the bulk-5-Fu measurements performed by Mazzola *et al.* [7] on crystalline silver – F : N : C : O= 0.9 : 2 : 4.2 : 1.6 (also normalized to N ≡ 2), strengthening the idea that such molecules can be thermally evaporated without too significant changes in the stoichiometry in a reproducible way.

Fig. 3 shows XPS measurements (in particular the F1s and N1s core levels) on a ∼ 1.1 nm 5-Fu film deposited on a polycrystalline sample. For comparison reasons, these results have been presented together with the data of 5-Fu on single crystal Ag reported in Ref. [7]. Note

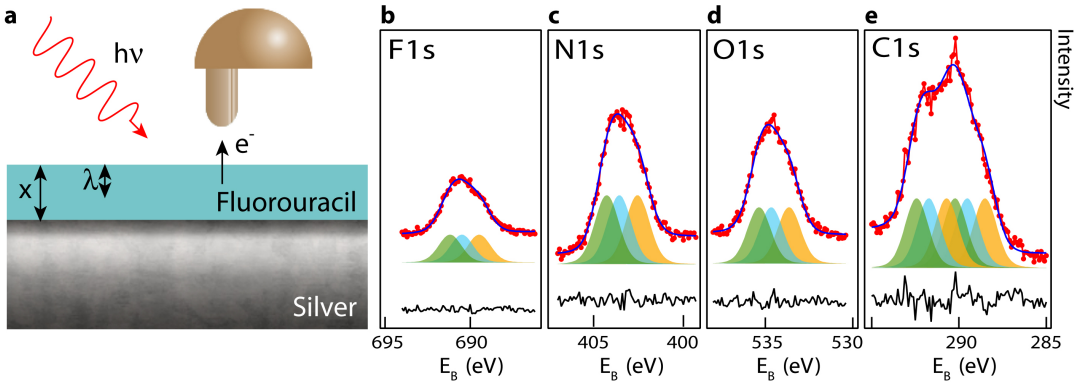

Figure 2. (a) Schematics of the XPS method. X-rays absorbed by the sample result in emission of photoelectrons. The electrons contributing to the signal are limited by their mean free path (λ). Since the thickness x of the 5-Fu film is significantly larger than the mean free path, $x \gg \lambda$, then the interface region is not probed. (b)–(e) XPS data containing photoelectron intensities of a bulk-like 5-Fu film (thickness $> 4\,\mathrm{nm}$) at low mobility ($-170\,^{\circ}\mathrm{C}$) showing the 1s core levels of F, N, O and C. Raw data (red dots) with fit model (blue curve) containing multiple components with separations $d_{\mathrm{green,\ blue}} \approx 0.7\,\mathrm{eV}$ and $d_{\mathrm{blue,\ orange}} \approx 1\,\mathrm{eV}$ for all core levels, indicating an inhomogeneous deposition. These bulk-measurements will act as a reference for determining if the 5-Fu molecule has degraded or not at body temperature.

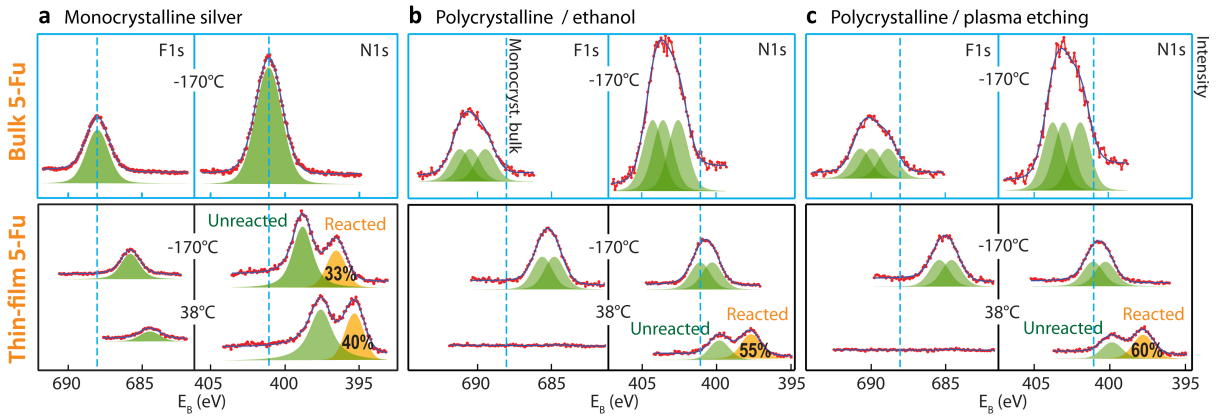

Figure 3. XPS measurements of the F1s and N1s core levels on (a) the monocrystalline Ag sample studied by Mazzola *et al.* [7], and the two polycrystalline Ag samples cleaned using (b) ethanol and (c) plasma etching. All peaks have been normalized to cross section, measuring time and beam current. A model (blue curve) made from Voigt-shaped components (green) has been fitted to the data (red dots). The two rows show the bulk-like 5-Fu (film thickness is $> 4\,\mathrm{nm}$) (bulk-like; upper panels) and $\sim 1.1\,\mathrm{nm}$ (thin-film; lower panels). The dotted line shows the location of the bulk peak center for the monocrystalline sample, to make the individual peak shifts more apparent. The similarity between the data from the two polycrystalline samples is in accordance with the similarity in contamination in Fig. 1. The "reacted" and "unreacted" N1s-components at body temperature are separated by $\sim 2.2\,\mathrm{eV}$ and $\sim 2.1\,\mathrm{eV}$ on the monocrystalline and polycrystalline samples, respectively, indicating that the reaction is also similar for all samples. The relative size of these N1s components indicates that respectively 80 % and 100 % of the 5-Fu molecules have reacted on each polycrystalline surface.

that all core levels obtained from the 5-Fu thin film are shifted compared to the corresponding bulk measurements. This can be attributed to a charge transfer between the 5-Fu and the substrate, which results in an effective doping of the system. The XPS data of Fig. 3 (a) and Fig. 3 (b), which correspond to 5-Fu on polycrystalline samples after ethanol cleaning and plasma etching, respectively, do not show significant differences. The samples, as mentioned above, have also a similar contamination level. Thus, hereafter, we will only consider data from the polycrystalline sample which has undergone a standard ethanol cleaning procedure. Conveniently, such a sample will be referred to as "the polycrystalline sample".

The data acquisitions in Fig. 3 (a) and reported in Ref. [7], show signs of a chemical reaction between the 5-Fu and the single crystal Ag substrate already at $-170\,^{\circ}\mathrm{C}$, indicating that the thermal energy needed for such reaction to take place is very small. This reaction manifests with an energy splitting (of ≈ 2.2 eV) of the N1s core level. Whilst at $-170\,^{\circ}\mathrm{C}$ no reaction is observed for

the polycrystalline sample, at body temperature the N1s of this system splits into two components, separated by 2.1 eV, a value comparable to the one inferred in Ref. [7] which might indicate a qualitatively similar reaction at this temperature. However, the different initial temperature needed for such a reaction to occur, suggests that the activation energy of this process might be different, hence it suggests a different reaction pathway.

In order to understand the mechanisms by which such a reaction takes place let us consider the stoichiometry of an intact 5-Fu molecule. Each 5-Fu molecule contains two N atoms. If all the 5-Fu molecules react and such a reaction occurs via one of the N atoms, in the photoemission signal we would expect a contribution to the N1s core level made of two equal peaks: one corresponds to the unreacted N atoms and one to the reacted one. Similarly, if only half of the molecules reacted, involving a change in the chemical environment of one of the N atoms, we can expect that the total signal to the N core level will be given by one peak (belonging the unreacted N) with 75% of the total N1s signal and one peak with the remaining 25% (reacted N atoms). According to the XPS spectra reported in Fig. 3 (b), 100% of the 5-Fu reacts at body temperature when in contact with the polycrystalline sample, while only 80% of the 5-Fu reacts on the monocrystalline model surface.

As reported in Fig. 3a and Ref. [7], on warming to body temperature, a loss of F occurs from 5-Fu on the monocrystalline Ag surface. This has been described in terms of desorption of HF. Similarly, the 5-Fu films on polycrystalline samples in Fig. 3 (b-c) exhibit a dramatic loss of F, whose signal at body temperature is completely extinguished. Given the similarity of the measurements on monocrystalline and polycrystalline samples, it is reasonable to assume that also a similar reaction takes place, but such a reaction seems to be stronger for the polycrystalline samples. In addition, the calculated ratio F : N is also very different for the thin 5-Fu film on the two samples at low temperature; F : N = 0.4 : 2 and F : N = 2.4 : 2 on the mono- and polycrystalline sample, respectively, both deviating from the stoichiometry of an intact molecule (F : N = 1 : 2). This all indicates that the reaction pathway may be different in the two cases, even though their reaction product (gaseous HF) is presumably the same.

Fig. 4 shows XAS data of the C and N K-absorption edge acquired for the 5-Fu thin film on the two polycrystalline silver substrates. The carbon data (Fig. 4 (a)) show only weak temperature dependence, except for the development of a small structure at low photon energies, at body temperature. Such a structure indicates that the

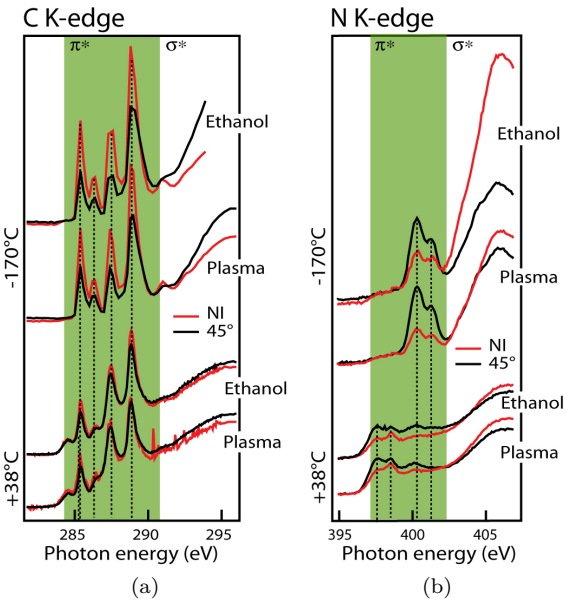

Figure 4. (a) XAS data showing the carbon K-edge for the 5-Fu thin film on the polycrystalline sample. The same π-structures are visible at $-170\,^{\circ}$C and $38\,^{\circ}$C, indicating an intact carbon-nitrogen ring. (b) Data on the N K-edge, where the appearance of additional structures at lower photon energy confirms the change in bonds configurations for some nitrogen atoms.

lowest occupied molecular orbital (LUMO) is at a lower energy configuration after heating the sample, indicating less occupied π-states, perhaps due to the loss of the F and H at body temperature. The existence of distinct π-bonds at both temperatures shows that the delocalized π-bonds remain, hence that the C and N ring is intact. The N K-edge (Fig. 4 (b)) is significantly modified with increasing thermal energy, and the arrival of two components located at lower photon energies at body temperature confirms that new nitrogen bonds are formed. The separation of these new components is similar to that one obtained on the original signal from N (~ 1 eV), and could thus correspond to the N atoms with new π-like bonding.

Both XPS and XAS are consistent with the picture that the reaction is probably similar to the one suggested in Ref. [7], showing that HF is released as a reaction product. However, the quantitative analysis of the XPS spectra shown in Fig. 3 indicates the possibility of an alternative reaction pathway for monocrystalline and polycrystalline samples. The pathway proposed in Ref. [7] for monocrystalline Ag sample can be summarized as follows: H and F are removed independently from a 5-Fu molecule and stick to the Ag surface already at low temperature. As the temperature is increased the H and F species become more mobile on the surface, thus they meet each other to finally form HF which desorps

from the surface into the vacuum. This mechanism explains why the 5-Fu molecule is seen to react already at a very low temperature, but the F is lost only when the temperature is increased. In the case of the polycrystalline sample, the 5-Fu molecules do not react at low temperature, and the F loss occurs simultaneously with a change in the N bonding configuration. This reaction can be regarded to a a single step process: one F and one H atom combine into HF, leaving the surface and going into the vacuum. Such a single step process would be consistent with the observation of C contamination in the polycrystalline sample. Indeed, C contamination can be expected to inhibit the catalytic activity of a surface, in this particular case, reducing the number of catalytic Ag sites. Thus, the C species would reduce the mobility of F and H, inhibiting the possibility for such species to move freely onto the Ag surface, meet and finally recombine, as observed instead in Ref. [7].

In order to get further insight into reaction pathway for 5-Fu on polycrystalline Ag, we performed density functional theory (DFT) calculations, revealing important details about the energy scales involved in this process. Importantly, we need to take into account the polycrystallinity of the Ag surface, as it is not obvious how such a polycrystallinity would influence the catalysis of different species. Whilst in the experiment the polycrystalline Ag surface is probably formed by several different crystal orientations and domains with vicinal surfaces, theoretically, we can reasonably approximate such a surface as formed by the three main Ag surface orientations Ag(111), Ag(100) and Ag(110). In Fig. 5 (a) we show a diagram of the activation energies per one 5-Fu molecule on the three main crystal surfaces of Ag mentioned. Such a diagram can be read out as map of the energy needed by one 5-Fu molecule to reach the nearest equilibrium situation, i.e. to make a step in the process. As visible in Fig. 5 (a), there are no significant differences for the reaction pathways on the differently oriented surfaces. This suggests that the polycrystallinity of our samples is not one of the major reasons behind the catalytic activity on the 5-Fu reaction.

Our calculations are also fully consistent with the idea that HF is released by the reaction between 5-Fu and the substrate. In addition by using DFT we can also guess which is the most favorable mechanism by which the reaction between 5-Fu and polycrystalline Ag happens. As already mentioned above, the presence of C contamination on the surface reduces the availability of catalytic Ag sites, thus making the reaction pathways on the polycrystalline sample different from the one found

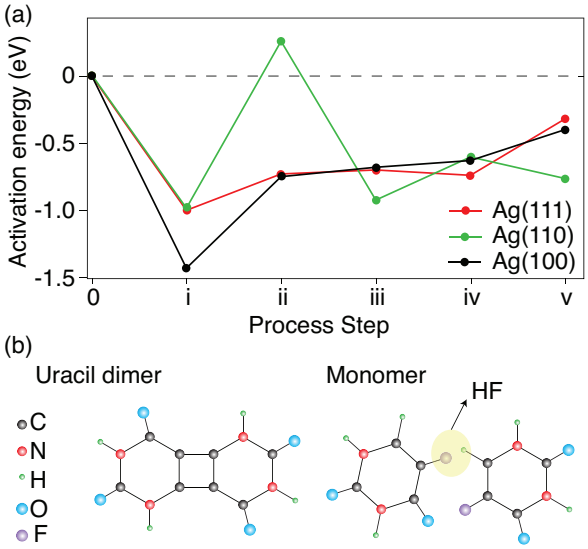

Figure 5. Energy of the reaction process and mechanism behind. (a) Activation energy diagram for the reaction between 5-Fu and different surface orientations. (b) Schematics of possible mechanisms by which HF is released from the reaction.

for a monocrystalline one. On these grounds, there might be two plausible ways, both forming HF as a reaction product, by which the reaction might occur. One way is that two 5-Fu molecules meet and form a dimer (see Fig. 5 (b)). Dimerization is a phenomenum which has important implications in biological processes: the formation of a squared C ring, caused by dimerization in molecules (pyrimidine) similar to the one here studied, is for instance the reason behind the generation of skin cancer cells [13]. Another way is that as soon as a F and a H atom from two different molecules are in contact, HF is formed and released into the vacuum. Although both scenarios are interesting and theoretically possible, the activation energy for monomers formation is much smaller than the one needed for forming dimers. This means that the formation of monomers is the most favorable process. Importantly, independently on the process pathways, in both cases HF is the reaction product.

In conclusion, XPS and XAS measurements show that 5-Fu degradation on silver surfaces is enhanced as the quality and cleanliness of the silver surface is reduced to that approximating real medical equipment. The intermediate reaction products due to degradation of 5-Fu are different on monocrystalline and polycrystalline substrates, but the final result is in both cases a plausible formation of hydrofluoric acid at body temperature. The fraction of reacted 5-Fu molecules indicates that the amount of HF produced by 5-Fu degradation could be even higher for medical quality silver than for a model surface. This is terrible news for medical applications, because it shows that the medical equipment

itself may unintentionally alter the function of the drugs, and that the millions of people receiving 5-Fluorouracil may experience additional suffering due to the general ignorance of silver-mediated HF production. Our results show that it is imperative to find a replacement for the silver in medical equipment. So far, high quality graphene remains the only surface that is known to leave the 5-Fu molecule intact, as described in the work by Mazzola *et al.* [7].

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